# SKRIPSI

# GENDER IS NOT A FACTOR IN THE ERYTHROMYCIN TOXICITY TEST USING *Oryzias javanicus* (Bleeker, 1854)

**Disusun dan diajukan oleh**

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**L021171011**

**PROGRAM STUDI MANAJEMEN SUMBER DAYA PERAIRAN**
**DEPARTEMEN PERIKANAN**
**FAKULTAS ILMU KELAUTAN DAN PERIKANAN**
**UNIVERSITAS HASANUDDIN**
**MAKASSAR**
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Sebagai Salah Satu Syarat Untuk Memperoleh Gelar Sarjana pada Fakultas Ilmu Kelautan dan Perikanan

**PROGRAM STUDI MANAJEMEN SUMBER DAYA PERAIRAN**
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## LEMBAR PENGESAHAN SKRIPSI

# GENDER IS NOT A FACTOR IN THE ERYTHROMYCIN TOXICITY TEST USING *Oryzias javanicus* (Bleeker, 1854)

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Telah dipertahankan di hadapan Panitia Ujian yang dibentuk dalam rangka Penyelesaian Studi Program Sarjana Program Studi Manajemen Sumber Daya Perairan Fakultas Ilmu Kelautan dan Perikanan Universitas Hasanuddin pada tanggal 22 November 2021 dan dinyatakan telah memenuhi syarat kelulusan

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Gender Is Not A Factor In The Erythromycin Toxicity Test Using *Oryzias Javanicus* (Bleeker, 1854)

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## ABSTRAK

**Andi Dina Hardiana**. **L021171011**. "Gender Is Not A Factor In The Erythromycin Toxicity Test Using *Oryzias javanicus* (Bleeker, 1854)" dibimbing oleh **Khusnul Yaqin** sebagai pembimbing utama dan **Irmawati** sebagai pembimbing pendamping.

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Eritromisin (ERY) adalah antibiotik yang banyak diresepkan dalam pengobatan manusia dan hewan untuk mengobati beberapa infeksi bakteri. Antibiotik ini dilaporkan memiliki berbagai efek pada lingkungan perairan. Untuk mengetahui apakah terdapat perbedan sensitivitas antara ikan *Oryzias javanicus* jantan dan ikan betina terhadap ERY, maka masing-masing lima ekor jantan dan ikan betina dengan panjang 3-3,5 cm dipapar dengan ERY dosis 2 $mg.L^{-1}$. Setiap individu ikan jantan dan ikan betina disimpan dalam wadah yang berbeda selama 96 jam. Parameter yang diukur adalah konsumsi oksigen dan tingkat kelangsungan hidup. Rata-rata konsumsi oksigen *O. javanicus* betina sebelum percobaan adalah 0,752 mg $O_2$ $g^{-1}$ $jam^{-1}$, berbeda nyata ($P<0,05$) dengan konsumsi oksigen *O. javanicus* jantan (0,474 mg $O_2$ $g^{-1}$ $jam^{-1}$). Setelah 96 jam terpapar ERY, konsumsi oksigen *O. javanicus* jantan pada akhir percobaan berkurang menjadi 0,353 mg $O_2$ $g^{-1}$ $jam^{-1}$ dengan tingkat kelangsungan hidup sebesar 8%, sedangkan, tingkat kelangsungan hidup *O. javanicus* betina adalah 0%. Penelitian ini menunjukan bahwa konsumsi oksigen *O. javanicus* jantan lebih rendah dibandingkan dengan ikan betina sehingga *O. javanicus* jantan yang terpapar 2 $mg.L^{-1}$ ERY lebih resisten dibandingkan dengan ikan betina. Namun demikian, secara statistik tingkat kelangsungan hidup ikan jantan dan ikan betina tidak berbeda nyata ($P<0,01$). Nilai $LT_{50}$-96 jam ikan *O. javanicus* jantan dan betina terhadap ERY sama yaitu 30 jam. Ikan jantan tidak lebih resisten terhadap ERY dibanding ikan betina. Penelitian ini menyimpulkan bahwa tidak ada perbedaan sensitivitas terhadap ERY antara *O. javanicus* jantan dan betina berdasarkan tingkat kelangsungan hidup dan $LT_{50}$-96 jam. Namun demikian dibutuhkan penelitian lanjutan dengan jumlah sampel yang lebih banyak yang dilengkapi dengan kontrol untuk mendukung hipotesa sementara tersebut di atas. Selain itu, perlu dilakukan pengecekan dan pemantauan keberadaan limbah antibiotik di perairan Indonesia untuk mencegah dan mengurangi adanya dampak pencemaran antibiotik di lingkungan perairan.



**Kata Kunci**: Eritromisin, antibiotik, perspektif gender, *Oryzias javanicus*, konsumsi oksigen, tingkat kelangsungan hidup

## ABSTRACT

**Andi Dina Hardiana**. *L021171011*. "Gender Is Not A Factor In The Erythromycin Toxicity Test Using *Oryzias javanicus* (Bleeker, 1854)" supervised by **Khusnul Yaqin** as a principle supervisor and **Irmawati** as co-supervisor.

Erythromycin (ERY) is an antibiotic widely prescribed in human and veterinary medicine to treat several bacterial infections. These antibiotics are reported to have various effects on the aquatic environment. To determine whether there was a difference in sensitivity between male and female *Oryzias javanicus* fish to ERY, each of five male and female fish with a length of 3 -3.5 cm were exposed to a dose of 2 $mg.L^{-1}$ ERY for 96 hours. Each male and female fish were stored in different containers during the study. The parameters measured were oxygen consumption and survival rate. The average oxygen consumption of female *O. javanicus* before the experiment was 0.752 mg O2 $g^{-1}$ $hour^{-1}$, significantly different ($P<0.05$) with oxygen consumption of male *O. javanicus* (0.474 mg O2 $g^{-1}$ body weight $hour^{-1}$). After 96 hour of ERY exposure, the oxygen consumption of male *O. javanicus* at the end of the experiment was reduced to 0.353 mg $O_2$ $g^{-1}$ body weight $h^{-1}$ with a survival rate was 8%, whereas, the survival rate of female *O. javanicus* was 0%. This study showed that the oxygen consumption of male *Oryzias* javanicus was lower than of female fish, so the male *Oryzias javanicus* exposed to 2 $mg.L^{-1}$ ERY was more resistant than female fish. However, statistical survival rates of male and female fish were not significantly different ($P<0.01$). The value of $LT_{50}$-96 hours both male and female *O. javanicus* fish to ERY was 30 hours. Males were not more resistant to ERY than females. This study concluded that there was no difference in sensitivity to ERY between male and female *O. javanicus* based on the survival rate and $LT_{50}$-96 hours. However, further research is needed with a larger number of samples equipped with controls to support the provisional hypothesis mentioned above. In addition, it is necessary to check and monitor the presence of the antibiotic waste in Indonesian waters to prevent and reduce the impact of the antibiotic pollutant on the aquatic environment.



**Keywords**: Erythromycin, antibiotic, gender perspective, *Oryzias javanicus*, oxygen consumption, survival rate.

## UCAPAN TERIMA KASIH

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Makassar, 26 November 2021

Penulis

## BIODATA PENULIS

Andi Dina Hardiana adalah anak pertama dari dua bersaudara, lahir pada tanggal 22 Oktober 1999 di Watampone, Kabupaten Bone. Penulis merupakan anak dari pasangan Bapak Pelda Baharuddin dan Ibu Rosdiana, S.Pd. Pada tahun 2011 penulis menyelesaikann sekolah dasar di SD Inpres 6/86 Biru. Tahun 2014 menyelesaikan sekolah menengah pertama di MTsN Watampone. Tahun 2017 penulis menyelesaikan sekolah menengah atas di SMAN 1 Watampone yang sekarang bernama SMAN 1 Bone. Pada tahun 2017 penulis diterima menjadi mahasiswa pada Program Studi Manajemen Sumber Daya Perairan Departemen Perikanan Fakultas Ilmu Kelautan dan Perikanan Universitas Hasanuddin melalui jalur Seleksi Nasional Masuk Perguruan Tinggi Negeri (SNMPTN).

Selama menjalani proses perkuliahan, penulis pernah menjadi asisten laboratorium Fisiologi Hewan Air. Pada bidang organisasi kemahasiswaan, penulis pernah menjabat sebagai Badan pengurus Harian (BPH) KMP MSP KEMAPI FIKP UNHAS periode 2019 sebagai sekretaris umum.

Penulis menyelesaikan rangkaian tugas akhir yaitu Kuliah Kerja Nyata (KKN) Tematik Bersatu Melawan Covid-19 Angkatan 104 di Kota Watampone, Kabupaten Bone pada tahun 2020. Penulis melakukan penelitian dengan judul "Gender Is Not A Factor In The Erythromycin Toxicity Test Using *Oryzias javanicus* (Bleeker, 1854)"

## KATA PENGANTAR

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Penelitian ini dilakukan sebagai salah satu syarat untuk memperoleh gelar sarjana di Fakultas Ilmu Kelautan dan Perikanan Universitas Hasanuddin. Penelitian ini dilaksanakan selama 2 bulan (Maret-April 2021). Adapun sumber dana penelitian ini berasal dari dana pribadi penulis. Penelitian ini telah dipresentasikan pada Symposium 8$^{th}$ National and 4$^{th}$ International Marine and Fisheries Faculty of Marine Science and Fisheries Universitas Hasanuddin pada 6 Juni 2021 dan tulisan ini sudah dimuat di IOP.(Hardiana, A.D., Yaqin, K., Yanuarita, D. and Tresnati, J., 2021, October. The effects of the antibiotic erythromycin on Oryzias javanicus in gender perspective. In *IOP Conference Series: Earth and Environmental Science* (Vol. 860, No. 1, p. 012011). IOP Publishing), sehingga penyusunan skripsi ini menggunakan bentuk skripsi model publikasi.

Penulis menyadari bahwa dalam tulisan ini terdapat banyak kekurangan. Oleh karena itu, kritik dan saran yang bersifat membangun sangat diharapkan penulis untuk kesempurnaan tulisan-tulisan kedepannya.

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Penulis

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# Gender Is Not A Factor In The Erythromycin Toxicity Test Using *Oryzias javanicus* (Bleeker, 1854)

## 1. Introduction

Antibiotics are antimicrobial drugs that function to kill or inhibit bacterial growth. Worldwide, the use of antibiotics reaches 100,000 tons per year (Danner *et al*., 2019). The use of antibiotics in large quantities has an impact on the environment. Antibiotics are drugs that are found as pollutants in water (Rodrigues *et al*., 2018). Antibiotics used to treat human and animal diseases are not fully absorbed by the body but almost 90% will be excreted together with urine and feces in an unchanged, still active form which can contaminate urban wastewater (Liu *et al*., 2020; Serwecinska, 2020). Unused or expired drugs also often flushed into the sewage system, which places an additional burden on the wastewater system and, thus, on the environment (Larsson, 2014; Serwecinska, 2020). Antibiotic residues enter the aquatic environment including river water through various pathways such as discharge of industrial effluent, hospital and municipal wastewater. Rivers appear to be a reservoir of antibiotic resistance and play an important role in transportation of antibiotic resistance between various environmental compartments. River water might create possible pathways for antibiotic resistance transmission between the environment, humans and animals (Hanna *et al*., 2020).

The introduction of antibiotics into the waters can pose serious threats to the environment such as the spread of bacteria and genes that are resistant to antibiotics. This can lead to an increase in the number of infections that are increasingly difficult to treat as the antibiotics used are less effective (Felis *et al*., 2020). Although antibiotics are specifically used to treat pathogenic infections (Danner *et al*., 2019), the introduction of antibiotics in water will have an impact on non-target organisms below 1 $\mu g.L^{-1}$ concentrations *(Pereira et al*., 2020). The presence of antibiotics continuously in the aquatic environment causes the organisms to be chronically exposed. In addition, active antibiotics even in low concentrations can cause toxic effects either because of their active ingredients or additives used in their formulations (Grenni *et al*., 2018).

One of the non-target organisms that can be affected by exposure to antibiotics is fish. In general, fish are considered the most likely organisms for environmental monitoring because they occupy the upper trophic level in the aquatic ecosystem as a carrier of low to high energy flow and absorption of drugs through the surface of the skin and gills carried by water or sediment (Rodrigues *et al*., 2018).

One of the most commonly used antibiotics is Erythromycin (ERY). ERY is a macrolide antibiotic that has been used since the 1950s to treat diseases in humans and is

also used in treating bacterial infections in fish farms. ERY is of particular concern in the aquatic field when compared to other antibiotics due to its use, release and persistence, and toxicity (Rodrigues *et al*., 2018). Research shows that ERY exposure can accumulate in the body of *Carassius auratus* fish and be maximally concentrated in muscle tissue (Liu *et al*., 2014). ERY can cause oxidative stress in fish, *Labeo rohita* (Renuka *et al*., 2019). Several studies have shown that ERY exposure can affect fish metabolism. Li & Zhang (2020) explained that a concentration of 2 $mg.L^{-1}$ ERYs can reduce the swimming speed of fish. Aerobic and anaerobic metabolic capacities were inhibited by ERY exposure. This suggests that ERY affects the energy metabolism of fish, specifically ATP synthesis and ATP release were inhibited, which inevitably damaged *D. rerio* and *O. latipes* (Li & Zhang, 2020).

Metabolism is all the organized chemical activities carried out by cells (Sanjana, 2019). Metabolic rate can be estimated by measuring the amount of oxygen consumed by living things per unit of time (Suharsono, 2018). Increased oxygen consumption indicates an increased metabolic rate in fish and indicates that the fish is experiencing stress so that it takes a lot of energy to adjust (Putra, 2015). Oxygen consumption is an important physiological parameter for assessing the toxicity of toxins because it is an important indicator of energy expenditure during metabolism (Tilak & Kumari, 2009). Fish that are exposed to toxic materials require large amounts of oxygen to detoxify these toxins (Li & Zhang, 2020).

It is important to consider sex as crucial in toxicological tests. This is because the distribution of contaminants in the fish body can differ between sexes in both behavior and physiology of fish (Madenjian *et al*., 2016). In such cases, the mercury concentration in muscle tissue and liver of *Petromyzon marinus* L showed that the mercury concentration in male fish muscle tissue was about 10% higher than that of female fish while the mercury concentration in the liver of female fish was about 60% greater than that of male fish (Pedro *et al*., 2014). The difference in accumulation will result in a difference in toxicity (Madenjian *et al*., 2016).

Based on this, ERY exposure can have an effect on the metabolic rate of fish which can be measured from the rate of oxygen consumption. Although there have been many studies on the effects of ERY exposure in fish, there is still less research on differences in fish gender sensitivity as measured by the level of oxygen consumption of male and female fish after exposure to pollutants, so this research needs to be carried out. The test fish used in this study was *Oryzias javanicus*, which widespread in Indonesia and this fish can live in freshwater and brackish water that has the potential to be a sentinel organism and is one of the test animals that are often used in ecotoxicology, but the information is still lacking regarding differences in gender sensitivity to pollutants such as antibiotics.

## 2. Materials and Methods

### *2.1. Chemical*

Erythromycin 500 mg was purchased at a local pharmacy. The ERY concentration used in the study refers to the research of Li & Zhang (2020), which was 2 $mg.L^{-1}$. The Erythromycin 2 mg is obtained using the following formula : desired dose / etiquette dose x weight (Suprapti, 2016) in 1 liter of water.

### *2.2. Fish sampling*

The sampling was carried out at the location of the Salarang River flow, Maros Baru District, Maros Regency, South Sulawesi. The fish were caught using a fishing net with a mesh size of 3 mm. The fish samples were put into a container that has been given an aerator and transferred to the laboratory. In the laboratory, 50 fish were selected, consisting of 25 male fish and 25 female fish with a length of 3 -3.5 cm.


Figure 1. *Oryzias javanicus* sampling location


Figure 2. *Oryzias javanicus* from the Salarang River

*2.3. The exposure experiment*

The collected of 50 fish *Oryzias javanicus* consisting of 25 male fish and 25 female fish with a length of 3-3.5 cm which were acclimatized in the laboratory. The fish were separated into two aquariums based on gender. Each aquarium was given aeration for maintaining oxygen supplies. This acclimatization aims to adapt to the physiological conditions of fish in the laboratory and was carried out for a minimum of 10 days *(Andriani et al.*, 2019) . During the acclimatization process, the fish were fed two times a day with the amount of feed 5% of the total body weight of the fish. The media were changed once a day to maintain water quality.

The ERY concentration used in the study refers to the research of Li & Zhang (2020), which was 2 $mg.L^{-1}$ (Li & Zhang, 2020). Five fish were used in each experimental unit in one liter of experimental media with five replications. The exposure was carried out for 96 hours. During the exposure process, the fish are fed once a day before changing the water. Observations were made every 6 hours. During the observation, the number of dead fish and water quality such as pH and temperature are recorded.

Measurement of oxygen consumption was carried out twice for male and female test animals before and after ERY exposure, using a respiration bottle. Oxygen consumption measurements after 96 hours of ERY exposure were carried out on fish that were still alive at the end of the study. After 96 hours of exposure, the fish were transferred to a glass jar without ERY exposure to measure their oxygen consumption. The dissolved oxygen measurement procedure in this study was carried out using the iodometric titration method.

Figure 3. The exposure experiment

*2.4. Research Variables*

2.4.1 *Oxygen consumption of fish*

Oxygen consumption of fish ($mgO_2$ $g^{-1}$ body weight $h^{-1}$) is estimated using the following formula:

$$\mathbf{OC = ( (DO_0 – DO_t)/ W x t) x V}$$

OC = oxygen consumption ($mgO_2$ $g^{-1}$ body weight $h^{-1}$), $DO_0$ = dissolved oxygen at the beginning of observation ($mgl^{-1}$) $DO_t$ = dissolved oxygen at the end of observation ($mg.L^{-1}$), W = weight of test fish (g), V = volume of water (l), t = time of observation (hours)

2.4.2. *Survival rate*

The survival rate is the percentage of the number of live fish and the number of fish at the end of the study. The survival rate (SR) calculation formula is: SR = Nt / No x 100%. SR = survival (%) Nt = the number of fish at the end of the study (individual) No = the number of fish at the beginning of the study (individual).

*2.5. Data analysis*

The student's t-test was used to determine differences in oxygen consumption of male and female fish. To analyze the relationship between length and weight on oxygen consumption, Pearson correlation analysis was carried out. Before the tests were performed, the data were first tested for normality and homogeneity. Data analysis was performed using Ms. application. Excel, GraphPad Prism 8, and SPSS 25.

## 3. Results and Discussion

The results of measuring the oxygen consumption of male and female *Oryzias javanicus* before exposure to ERY 2 $mg.L^{-1}$ found that there was a statistical difference in oxygen consumption between male and female *Oryzias javanicus* and female *Oryzias javanicus* ($p < 0.05$).

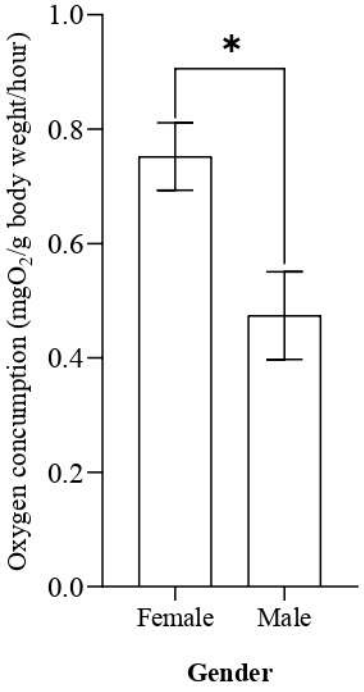


Figure 4. Comparison of oxygen consumption of male and female *Oryzias javanicus* before exposure to ERY. * Asterix showed a statistically significant difference ($P < 0.05$).

The average oxygen consumption of female *Oryzias javanicus* was 0.752 $mgO_2\ g^{-1}$ body weight $h^{-1}$, while the average consumption of male Oryzias javanicus was 0.474 $mgO_2\ g^{-1}$ body weight $h^{-1}$.The results of statistical tests using Student's t-test analysis showed that oxygen consumption between male and female *Oryzias javanicus* was significantly different ($P < 0.05$). This shows that the oxygen demand for female fish is more than that of male fish. The average total length of male *Oryzias javanicus* was 3.24 cm and the average body weight was 0.35 g, meanwhile the average total length of female *Oryzias javanicus* was 3.23 cm and the average body weight was 0.34 g. It was known that the male and female of *Oryzias javanicus* were adult fish that have matured gonads (Ibrahim *et al*., 2021). This is thought to be related to the higher energy requirements of female *Oryzias javanicus* compared to male *Oryzias javanicus*, because female *Oryzias javanicus* fish are preparing to produce prime quality eggs with thick chorions as a consequence of the K strategy.

The relationship between length, weight, and oxygen consumption of male and female *Oryzias javanicus* fish can be seen in Table 1. From the results of the correlation analysis using the Pearson correlation analysis, it was known that the correlation between weight and oxygen consumption of male fish was -0.5046, and the correlation between length and oxygen consumption of male fish was -0.6100. The correlation between weight and oxygen consumption of female fish was -0.2886, and the correlation between weight and oxygen consumption of female fish was -0.3565 From the results of statistical analysis, it was found that there was no correlation between oxygen consumption and weight of male fish (p=0.3860 and length (p=0. 2746) and there was no correlation between oxygen consumption and weight of female fish (p=0.6377) and length (p=0,5559). This means that oxygen consumption for adult fish is not affected by weight and total length.

Table 1. The correlation between length and weight of fish and oxygen consumption

| Correlation | Male | | Female | |
|---|---|---|---|---|
| | Oxygen Consumption | | Oxygen Consumption | |
| | R | P value | R | P value |
| **Weight** | -0,5046 | 0,3860 | -0,2886 | 0,6377 |
| **Total Length** | -0,6100 | 0,2746 | -0,3565 | 0,5559 |

After exposure to ERY 2 $mg.L^{-1}$ for 96 hours, it was found that the oxygen consumption of male fish was 0.353 $mgO_2$ $g^{-1}$ body weight $h^{-1}$. This value indicates that the oxygen consumption of male fish after exposure is lower than before exposure, namely 0.474 $mgO_2$ $g^{-1}$ body weight $h^{-1}$. Meanwhile, all-female fish experienced total death at the end of the period. There was a decrease in oxygen consumption in male *Oryzias javanicus* and total mortality in female *Oryzias javanicus* after exposure to ERY antibiotics. These were presumably because ERY enters the fish's body more through the gills than other pathways such as skin so that it can interfere with the performance of the gills which were the respiratory organs. This disturbance ultimately resulted in death through an increase in the amount of mucus in the gills and a decrease in the rate of oxygen consumption (Juanda & Edo, 2018; Puspitasari *et al.*, 2018; Rodrigues *et al.*, 2018).

Based on the results of the study, it was found that male *Oryzias javanicus* fish experienced a decrease in the survival rate to 92% in 6 hours. Meanwhile, the female experienced the same decrease in survival rate as the male fish in the 12 hours. However, after entering the 48 hours it was found that the survival rate of female fish was lower than that of male fish and continued to decline, resulting in total mortality in the 72 hours. Male fish can survive until the end of the 96 hours with a survival rate of 8%. The same decrease in survival rate at different periods between these fish indicates that male fish were more sensitive in the early period to ERY contaminants than female fish. However, at longer periods, female fish become more sensitive than male fish.

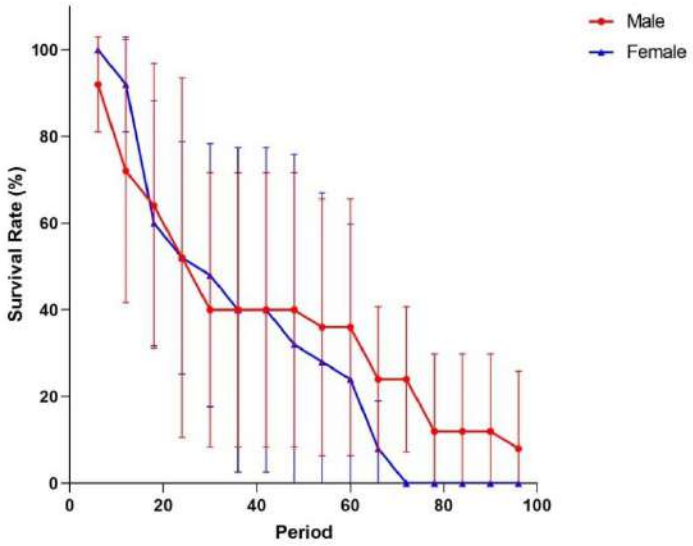


**Figure 5.** The survival rate of male and female *Oryzias javanicus*.

The results of the current study were consistent with research by Amal *et al*., (2018) that revealed *Oryzias javanicus* experienced mortality in the fourth period after being exposed to *Streptococcus agalactiae* while female fish only experienced mortality in the sixth period. Liu *et al*., (2014) who showed differences in sensitivity in *Oryzias latipes* that male fish were more sensitive than female fish to exposure to 2,4,6-trichlorophenol. This was in contrast to the current study which showed no significant difference between the percent mortality of male and female animals.

ERY is a macrolide antibiotic. Macrolide antibiotics can cause DNA damage through the induction of oxidative stress in fish. The SOD activity in the gill of adult zebrafish was slightly higher treated by antibiotic maduramicin which organ reaction to maduramicin in terms of oxidative stress indicate that this antibiotic have effect on antioxidant enzyme of the gill of zebrafish (Ni *et al*., 2019). ERY also causes significant changes in gill with acute or chronic exposure (Yang *et al*., 2020). Exposure to ERY at 0,1 $\mu g.L^{-1}$ delayed hatching and decrease the survival rate of Zebrafish (*Danio rerio*) effect on disturbed mitochondrial function (Minski *et al*., 2021). This research is also supported by Zhao (2016) research that the activity of antioxidant enzymes in the liver, namely Superoxide Dismutase (SOD) and catalase (CAT), increases in females. This mechanism can reduce the chemical content to maintain homeostasis. As the main defense system against exterior compounds, SOD and CAT activity play an important role in antioxidant defense. Inhibition of SOD and lack of CAT response indicates some susceptibility to the antioxidant system and oxidative stress in organisms caused by pollutants. SOD activity was significantly induced in the female *Oryzias melastigma* group during the initial period of sulfamethazine exposure. Meanwhile, male *Oryzias melastigma* showed no significant increase in SOD induction at all concentrations of

sulfamethazine exposure. Male *Oryzias melastigma* at a period of two hours showed a slower response

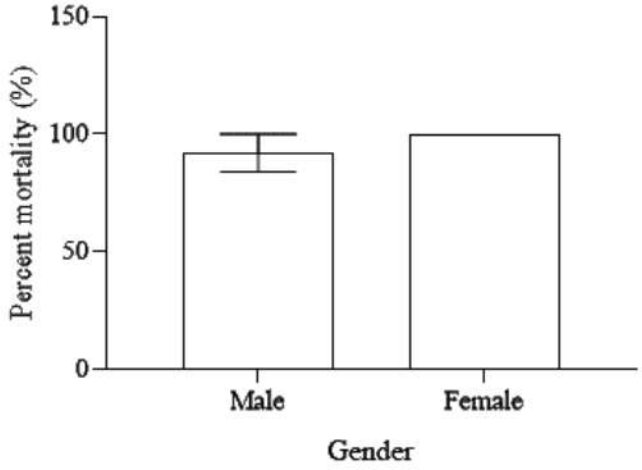


**Figure 6.** The percent mortality of male and female *Oryzias javanicus*.

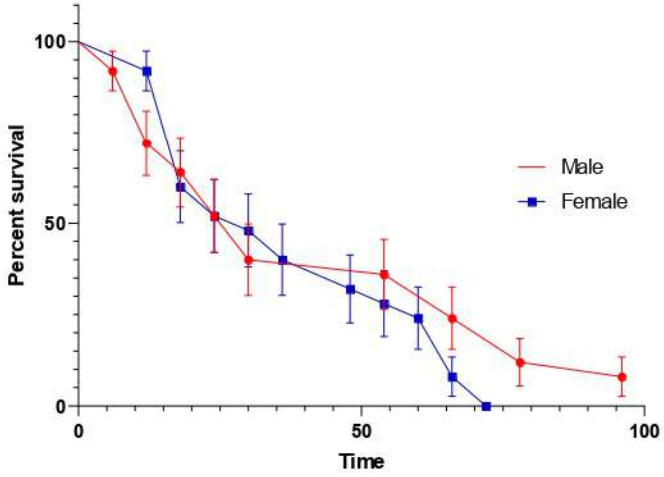


**Figure 7.** $LT_{50}$-96 hour of male and female *Oryzias javanicus*.

than female fish. Hence, male fish are more susceptible than female fish to chemical exposure in the early period. However, for CAT activity, there was a decrease within 12 hours in female fish. The activity of this antioxidant enzyme also affects the time and concentration of exposure. SOD activity in males was induced at high doses. It can be found that at a concentration of 40µg.$L^{-1}$ male fish had the highest metabolites while female fish at a concentration of 40µg.$L^{-1}$ had liver damage and decreased activity of Cat in 24 hours and CAT inhibition in the next section. This shows that the liver of female fish is more susceptible than male fish. This is because the excessive accumulation of sulfamethazine in the fish liver causes inhibition of CAT function (Zhao *et al*., 2016). But, the results of statistical analysis